

411.7

SILICA-INDUCED NUCLEAR FACTOR- κ B ACTIVATION: INVOLVEMENT OF REACTIVE OXYGEN SPECIES AND PROTEIN TYROSINE KINASE ACTIVATION.

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Nuclear Factor- κ B (NF- κ B) is a multiprotein complex that may regulate a variety of inflammatory cytokines involved in the initiation and progression of silicosis. The present study documents the ability of in vitro silica exposure to induce DNA-binding activity of NF- κ B in mouse macrophages (RAW264.7 cells) and investigates the role of reactive oxygen species (ROS) and/or protein tyrosine kinase in this activation. In vitro exposure of mouse macrophages to silica (100 μ g/ml) resulted in a 2-fold increase in ROS production, measured as the generation of chemiluminescence (CL), and caused activation of NF- κ B. Silica-induced CL was inhibited 100% by superoxide dismutase (SOD) and 75% by catalase, while NF- κ B activation was inhibited by a variety of antioxidants (catalase, SOD, sodium formate, α -tocopherol, and pyrrolidine dithiocarbamate). Further evidence of the involvement of ROS in NF- κ B activation is that 1 mM H₂O₂ enhanced NF- κ B/DNA binding and that this activation was inhibited by catalase. Specific inhibitors of protein tyrosine kinase, such as herbimycin A, genistein and AG-494, prevented NF- κ B activation in silica-treated cells. Genistein and AG-494 also prevented NF- κ B activation in H₂O₂-treated cells. In contrast, inhibitors of protein kinase A or C, such as H89, staurosporin, calphostin C, chelerythrine, and H7 had no inhibitory effect on this response. The results suggest that ROS play a role in silica-induced NF- κ B activation in macrophages and that phosphorylation events mediated by tyrosine kinase may be involved in this activation.

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ENHANCED PRODUCTION OF PROSTAGLANDIN E₂ (PGE₂) BY ALVEOLAR MACROPHAGE (AM) HARVESTED FROM SILICA-EXPOSED RATS: POSSIBLE REGULATORY ROLE OF NITRIC OXIDE (NO). JYC Ma, H-M Yang, MW Barger, JKH Ma and V Castranova. HELD, NIOSH and Sch. of Pharmacy, West Virginia Univ., Morgantown, WV.

This study investigated whether NO plays a significant role in regulating the production of PGE₂ or inflammatory cytokines by silica-exposed AM. Rats were treated by intratracheal instillation of saline (control) or silica (20 mg/rat) and AM harvested by bronchoalveolar lavage at 1, 3, 7, 14, and 28 days post-exposure. AM were cultured for 24 hr and ex vivo production of PGE₂, leukotriene B₄ (LTB₄), TNF- α , IL-1, and NO were monitored. In vivo exposure to silica resulted in an increase in PGE₂ production which peaked (185 fold) at 3 days post-exposure, while LTB₄ release was decreased. Silica exposure increased NO production by 9 fold at 3 days post-exposure, while causing a moderate increase in TNF- α production and a significant elevation of IL-1 secretion which was sustained through 28 days post-exposure. Tetrandrine, an antifibrotic agent, inhibited IL-1 and TNF- α production but did not affect NO or PGE₂ release. Indomethacin blocked PGE₂ production completely and had a moderate effect on NO production. Furthermore, inhibition of NO production with N-nitro-L-arginine methyl ester enhanced PGE₂ production without affecting TNF- α or IL-1 release. These results suggest that silica-induced stimulation of PGE₂ production is controlled via a different pathway than cytokine production and that NO may play a role in limiting PGE₂ production in silica-exposed rats.

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Effects of Peroxynitrite on the Pulmonary Oxidative State and Vascular Permeability. D.L. Beckman, P. Mehta, V. Hanks, W.H. Rowan and L. Liu. Department of Physiology, East Carolina Univ. Greenville, NC 27858

Excess nitric oxide (NO) formation especially in the presence of superoxide anion (O₂⁻) leads to the formation of peroxynitrite (OONO⁻) which may result in lung injury. Oxidant-mediated lung injury has a critical role in pulmonary diseases. We therefore determined whether OONO⁻ affects pulmonary vascular permeability, lipid peroxidation and formation of nitrotyrosine using an isolated perfused rat lung model. The lung weight gain during bolus OONO⁻ infusion increased in a dose-dependent manner over a range of 3-30 μ moles. OONO⁻ increased the production of thiobarbituric acid reactive substances, an index of lipid peroxidation. Furthermore, nitrotyrosine levels in lung tissue rose with increased concentration of OONO⁻ as determined by western blot using anti-nitrotyrosine antibodies. These results suggest that OONO⁻, formed from NO and O₂⁻, leads to increased pulmonary vascular permeability possibly through lipid peroxidation and/or nitration of cell membrane proteins. (Supported by the UNC Institute of Nutrition and NIH 52146).

ISCHEMIA/REPERFUSION INJURY (412.1-412.2)

412.1

TNF α REQUIRES KUPFFER CELLS TO INITIATE HEPATIC INJURY FOLLOWING HINDLIMB ISCHEMIA IN THE RAT.

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We tested the hypothesis that TNF α indirectly initiates hepatic injury through the activation of Kupffer cells following a remote ischemic insult. We applied intravital fluorescence microscopy to the livers of normotensive rats following 4-hrs of hindlimb ischemia/reperfusion (I/R). After 3-hrs of reperfusion, hepatic injury was determined based on measures of lethal hepatocyte injury (#/10² mm²) using propidium iodide (0.05 mg/100g BW), and the number of continuously perfused sinusoids (% of total sinusoids). Rats were randomly allocated to test the role of Kupffer cells (n=4), TNF α (n=4), or the interaction between TNF α and Kupffer cells (n=4) by: i) a pretreatment with gadolinium chloride 24-hrs prior to ischemia (GdCl₃; 1 mg/100gBW); ii) a polyclonal antibody against TNF α administered prior to reperfusion (3.5x10⁴ neutralizing units/100gBW); iii) or a combination of the treatments. Sham rats (n=5) received limb ischemia only. Systemic TNF α , measured by ELISA, peaked at 30 minutes of reperfusion (60.5 \pm 22.2 pg/ml), returning to baseline within 90 minutes. In all treatment groups, injury was reduced at least 2-fold (Sham: 158.5 \pm 21.1 vs. TNF α PAB: 61.8 \pm 32.4 vs. GdCl₃: 59.7 \pm 21.7; p<0.05). The reduction of Kupffer cell function, or the inhibition of TNF α resulted in a 15-20% increase in the number of continuously perfused sinusoids, with no appreciable difference observed between treatments. No additional benefits were provided by the combination of treatments. In addition, TNF α , in the absence of Kupffer cell function, did not appear to initiate significant hepatic parenchymal or microvascular injury. These results suggest that TNF α requires Kupffer cells to initiate remote hepatic injury following limb ischemia. Supported by MRC Canada & LHSC.

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PRESENTATION SIGNS IN RATS AFTER 10-MINUTES TOTAL ISCHEMIA. N. POPOVA, L. TEL, E. DALENOV
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Complex of pathologic and compensatory reactions after 10-minutes total ischemia leads to development of organism presentation.

Investigations on the white rats one year after total ischemia revealed decrease of motional and orientative-investigative activity, that of ability for memorizing of trined reflexes and their preservation, decrease of muscular strength. Besides cholesterol accumulation in the important living organs and tissues, development of atherogenesis dislipidemia, oxidative modification of lipoproteins were observed.

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ABSTRACTS
PART I

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